C_1 Compounds as Substrates for the Production of Single-Cell Protein

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The cultivation of micro-organisms that utilize methane and/or methanol as their carbon energy sources for growth will be discussed. Emphasis will be placed on the methods employed for increasing the yield of protein-rich biomass and for improving the productivity of the systems employed. Some experimental results obtained in laboratory-scale fermenters will be reviewed.

Allosteric Binding of Coenzymes to Alcohol Dehydrogenases

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NAD^+ or $NADP^+$ preparations have been shown to accelerate the thermal inactivation of both yeast and liver alcohol dehydrogenases during incubation, before assay, for 20 or 50min respectively at 43°C at pH 8.8 (Wiseman, Hanley & Williams, 1971).

This effect was concentration-dependent in the range $1\frac{1}{2}$–4mM approximately; 50% recovery, of the activity obtained after heating in the absence of NAD^+ or $NADP^+$, was found at about $2\frac{1}{2}$mM-NAD^+ or -$NADP^+$. This suggests a K_d of this order for some other binding site present, because the catalytic site of alcohol dehydrogenases shows a much lower K_m for these coenzymes. For example the K_m of liver alcohol dehydrogenase for NAD^+ was reported to be 51μM at pH 8 (Theorell & McKinley-McKee, 1961). Clear evidence for other such additional binding sites has been reported (Weiner, 1969). Each molecule of liver alcohol dehydrogenase (two subunits/molecule) bound a spin-labelled coenzyme analogue (an inhibitor competitive with NAD^+) at two strong ($K_d = 17 \pm 8\mu$M) and five or six somewhat weaker sites ($K_d = 75 \pm 9\mu$M) in metal-free phosphate buffer at pH 6.0. Also, NAD^+ has been found to quench the fluorescence of NADH bound to liver alcohol dehydrogenase (Theorell & McKinley-McKee, 1961).

The thermal inactivation of alcohol dehydrogenases promoted by NAD^+ or $NADP^+$ preparations could be due to a conformational change caused by allosteric binding of these coenzymes, with relatively weak affinity for that site. Recent work here (A. Wiseman, unpublished work) with yeast alcohol dehydrogenase bound to DEAE-cellulose (Miles–Seravac Ltd., Maidenhead, Berks., U.K.) has demonstrated a much higher thermal stability for this bound enzyme as compared with free enzyme. This followed the same preincubation treatment, with or without NAD^+ as appropriate, followed by the addition of ethanol, then rapid shaking for 2min and immediate filtration and spectrophotometric determination at 340nm (the K_m for NAD^+ was found to be about 3 times that of the free enzyme). The allosteric site for thermal inactivation by coenzyme may be blocked during the binding procedure in this case.

Theorell, H. & McKinley-McKee, J. S. (1961). *Acta chem. scand.* **15**, 1811.
Weiner, H. (1969). *Biochemistry, Easton*, **8**, 526.
Wiseman, A., Hanley, H. O. & Williams, N. J. (1971). *Biochem. J.* **123**, 8P.

Quaternary Structure of Oligomers: a New Theory and its Application

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This approach to the study of self-assembly of proteins with identical globular subunits coordinates and extends current ideas about symmetry in protein structures (Klug, 1967) with the concept of isologous and heterologous association (Monod, Wyman & Changeux, 1965). In the simplest possible type of self-assembly a subunit or protomer is assumed to contain two corresponding, but different, sites on its surface. It is proposed that any two such sites on a protomer are called a 'site-pair'. It is shown that the quaternary structure of almost all proteins may be described in terms of only a few simple binding patterns characterized by the number of site-pairs on each protomer and by their isologous or heterologous nature. The geometry of multisubunit proteins is further clarified by defining four types of angle, called θ, ϕ, ϵ and ζ, which are parameters of the arrangement of the binding sites on each subunit. This present communication is limited to a consideration of oligomers.

Three main types of oligomer assembly can be distinguished: the protomer has, in each case, (*a*) one site-pair, (*b*) two isologous site-pairs and (*c*) one isologous and one heterologous site-pair. The range of possible structures is shown to correspond to proteins with respectively (*a*) cyclic symmetry (mostly dimers), (*b*) dihedral symmetry and (*c*) cubic symmetry. [Exceptions to this scheme should be noted but seem less likely. Dihedral symmetry is possible with the type of binding in class (*c*), and cubic symmetry may be generated from protomers